



# Attack, Defend and Persist: How the Fungal Pathogen *Candida auris* was Able to Emerge Globally in Healthcare Environments

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**Abstract** Within a decade after its first description, the multidrug-resistant yeast *Candida auris* has emerged globally as a nosocomial pathogen causing difficult to control outbreaks. This, together with the alarmingly high mortality rate of up to 66% associated with *C. auris* candidemia, calls for a better understanding of its virulence traits and routes of transmission. Unlike other clinically relevant *Candida* species, *C. auris* seems to have the unique ability to be easily transmitted between patients. Although initially thought to express fewer virulence traits than *Candida albicans*, recent genomic insights suggest *C. auris* to possess these traits to a much more similar extent. This review highlights the virulence traits *C. auris* expresses to attack the host, defend itself against antimicrobial agents and to persist within the healthcare environment.

**Keywords** *Candida auris* · Virulence traits · Pathogenicity · Genomics · Disinfectants · Phenotypic switch · Morphogenesis · Biofilms · Resistance · Animal models

## Introduction

Annually, an estimated 1.5 million people die from invasive fungal infections [1]. The advance of life expectancy, the rise of immunosuppressive treatments, higher survival of patients living with cancer or chronic disease and the use of catheters are all factors that attributed to the emergence of opportunistic fungal pathogens over the last decades [2, 3].

*Candida* species are considered the most frequent fungi encountered in hospital settings accounting for more than 400,000 cases of bloodstream infections each year, making them the third to fourth most common cause of invasive fungal infections worldwide [1, 4–6]. *Candida albicans* is recognized as the main causative pathogen of candidiasis [1]. However, new species are on the rise, with the globally emerging multidrug-resistant *Candida auris* as one of the most concerning examples. Treatment options of *C. auris* are limited due to antifungal resistance, misidentification and its ability to persistently colonize hospital environments.

Since its first description in 2009, *C. auris* has been reported in over 25 countries on five continents (Fig. 1), causing fungemia outbreaks with crude

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mortality rates ranging from 32 to 66% [7–9]. Because the *Candida* genus is composed of a highly heterogeneous group of species, *C. auris* differs markedly from common well-studied pathogenic *Candida* species such as *C. albicans* and *C. glabrata* [10, 11]. For example, where other *Candida* infections are thought to result from autoinfection from host flora, *C. auris* seems to have the unique ability to persistently colonize the host skin, making it easily transmissible between patients [10, 12]. This transmissibility together with its multidrug resistance and high mortality rates makes *C. auris* a serious threat to public health.

A large number of papers have addressed the epidemiology of *C. auris* (reviewed by [8, 13, 14]). However, its mechanisms of pathogenicity and virulence have only recently been discussed [15]. Here, we will further elaborate on the current knowledge of virulence traits of *C. auris*, highlighting the differences and similarities with the most common pathogenic *Candida* species helping to understand how *C. auris* was able to emerge rapidly as a new global nosocomial pathogen.

#### What the Genome of *C. auris* is Telling Us

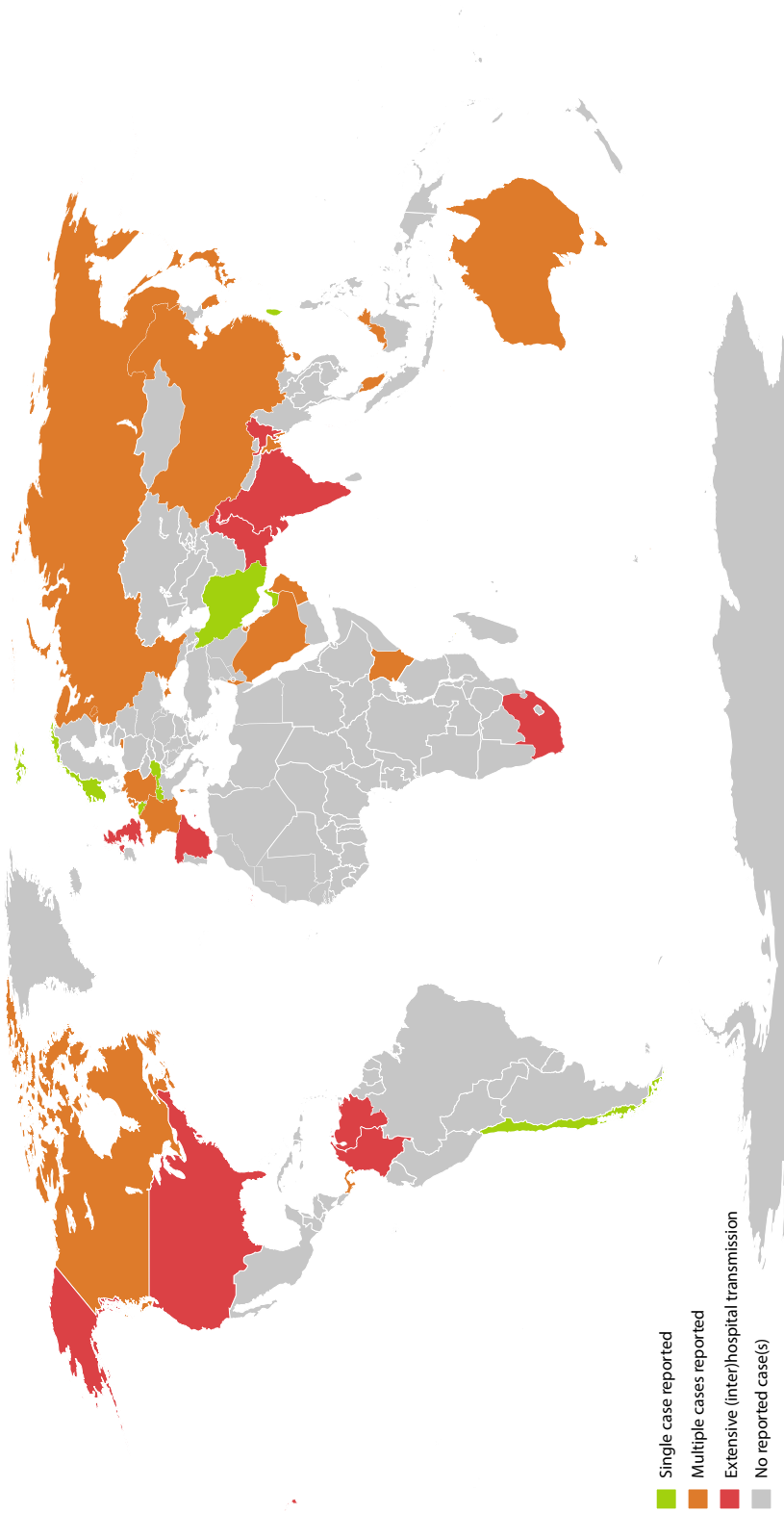
In 2009, *Candida auris* was described as a novel species belonging to the *C. haemulonii* species complex (*Metschnikowiaceae* clade), after isolation from the ear canal of a 70-year-old Japanese woman [16]. Unfortunately, *C. auris* is often misidentified as *C. haemulonii*, *Candida famata* and *Rhodotorula glutinis* by commercial biochemical identification systems such as Microscan (Beckman Coulter, Pasadena, CA) and API-20C AUX (BioMérieux, Marcy L'Etoile, France). Therefore, systems using matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) or identification by sequencing the internal transcribed spacer region (ITS) are needed for rapid and reliable identification of this yeast [17, 18]. Previously, lack of *C. auris* entries in the libraries made it impossible for both the Bruker biotyper (Bruker Daltonics, Bremen, Germany) and VITEK-MS (BioMérieux) MALDI-TOF MS systems to identify *C. auris*. However, when queried on an updated database, containing *C. auris*, accurate identification is possible [19].

A retrospective review of *Candida* isolates from three reported cases of nosocomial fungemia in South

Korea showed that all cases were caused by *C. auris*, with the earliest report dating back to 1996 [20]. However, it remains unclear why *C. auris* has only recently emerged globally as a nosocomial pathogen. Whole genome sequencing (WGS) of clinical isolates led to the description of four unique clades of *C. auris* divided by geographic region (East Asian, South Asian, South African and South American). WGS analysis has shown that thousands of single-nucleotide polymorphisms (SNPs) separated clades, but within clades isolates are clonal. This analysis indicates the independent, nearly simultaneous emergence of clonal populations of *C. auris* on three continents [7].

The genomes of many pathogenic *Candida* species, such as *C. albicans* and *C. glabrata*, have extensively been studied, while the high-quality genome of *C. auris* was only very recently investigated [21–24]. Despite the recent efforts made in sequencing the *C. auris* genome, detailed information regarding the genome architecture, virulence and multidrug resistance of *C. auris* is lacking. Muñoz and colleagues (2018) used high-quality genome data to investigate the interspecies relationships and observed that within the *C. auris* clade the average pairwise nucleotide identity was 98.7% and that the nucleotide genome identity between *C. auris* and each of the siblings *C. haemulonii*, *C. duobushaemulonii* and *C. pseudo-haemulonii* was 88% [24]. In addition, 40% of the predicted proteins were found to be orthologous to those of *C. lusitanae* [23]. Nonetheless, the majority of these proteins are uncharacterized and hypothetical proteins. Therefore, it remains unclear whether these proteins are involved in the highly virulent and pathogenic behavior of *C. auris*.

Comparison of the *C. auris* genome to the more well-annotated and well-studied genome, yet distantly related, of *C. albicans* suggested the presence of a significant number of orthologues that may contribute to the virulence of *C. auris*. This set of orthologues included transporters belonging to the major facilitator superfamily and ABC (ATP binding cassette) superfamily, secreted proteinases, lipases, phospholipases and aspartyl proteases [21, 23]. However, the roles of specific genes need to be further investigated. The *C. auris* genome was also found to encode for kinases like Hog1, Protein Kinase A (PKA) and two-component histidine kinase which have been reported to be involved in the regulation of stress signaling pathways to enhance tolerance of pathogenic fungi to chemical



**Fig. 1** Global epidemiology of *Candida auris*. Updated map of the *C. auris* epidemiology based on data presented by the Centers of Disease Control (Atlanta, GA, USA; <https://www.cdc.gov/fungal/candida-auris/tracking-c-auris.html>; [18]). Single (imported) cases were reported from Austria, Belgium, Chile, Iran, Norway, Switzerland, Taiwan and the United Arab Emirates. Multiple cases of *C. auris* infections were reported from Australia, Bangladesh, Canada, China, France, Germany, Israel, Japan, Kenya, Kuwait, Malaysia, the Netherlands, Oman, Panama, Russia, Saudi Arabia, Singapore and South Korea. From Colombia, India, Pakistan, South Africa, Spain, the UK, the USA and Venezuela extensive (inter)hospital transmissions were reported

fungicides [25]. Hog1 has recently been shown to play a role in regulating stress resistance, cell morphology, aggregation and virulence in *C. auris* [26]. The function of other kinases in *C. auris* still needs to be experimentally verified.

### The Many ‘Faces’ of *C. auris*

Phenotypic switching and morphogenesis are key features for virulence of *C. albicans* and therefore extensively studied in this species (as reviewed by others [27–29]). *C. albicans* deploys two typical switching systems, namely the morphological yeast-filament transition and phenotypic white-opaque switching. The ability to produce hyphae is thought to promote virulence by giving the fungus the ability to invade epithelial cell layers by exerting mechanical force, breaching and damaging endothelial cells, and causing lysis of macrophages and neutrophils following phagocytoses [27].

The first micromorphological studies of *C. auris* suggested that this pathogen was not capable of forming germ tubes, pseudohyphae or hyphae [20, 30, 31]. However, pseudohyphae-like forms seem to be induced under high-salt stress and when forming biofilms. These pseudohyphae are characterized by rudimentary growth, elongated shape and incomplete cell division [31, 32]. The presence of a pseudohyphal phenotype in *C. auris* suggests the potential to undergo filamentation under certain unidentified conditions. Although well-known environmental factors of filamentation in *C. albicans* could not induce filamentous growth in *C. auris* in vitro [31], Yue et al. [33] showed that passage of *C. auris* through a mammalian host could trigger a heritable switch between the typical yeast and a filamentous phenotype, in which hyphae are formed. *C. auris* cells recovered from mice kidney and liver tissues displayed very elongated filaments after culturing on YPD medium for 24 h. More recently, a regulative role of the essential molecular chaperone Hsp90 in the morphogenesis of *C. auris* was found. Depletion of Hsp90 in *C. auris* resulted in filamentous growth similar to *C. albicans*, showing Hsp90 to be a key regulator of morphogenesis [34].

Comparative genomics showed genes encoding homologs of *C. albicans* hyphal regulators to be upregulated in filamentous cells of *C. auris* [33]. On the other hand, a number of conserved genes that govern filamentous growth in *C. albicans* are

differentially expressed in *C. auris*. For example, *EFG1* known to be required for filamentous growth in *C. albicans* is down regulated in filamentous *C. auris* cells [33, 35]. Moreover, genome analysis has shown that *C. auris* lacks the gene encoding candidalysin (*ECE1*) and hyphal cell wall protein (*HWPI*), both of which are strongly associated with hyphal formation [24, 34]. This suggests a partially conserved mechanism for filamentation between *C. auris* and *C. albicans*, but also the presence of other transcriptional regulators and signaling molecules, capable of inducing filamentation in *C. auris*, activated by yet to be explored environmental factors.

Phenotypic switching has been described in many *Candida* species such as *C. albicans* [36], *C. glabrata* [37], *C. tropicalis* [38, 39] and *C. dubliniensis* [40]. The reversible and heritable switching between white and opaque cellular phenotypes is a well-known virulence attribute of *C. albicans*, but also plays a role in sexual reproduction [36]. This phenotypic plasticity allows the fungus to colonize distinct host niches. For example, opaque cells show reduced virulence compared to white cells, but are excellent colonizers of the skin. Moreover, opaque cells are phagocytosed less efficiently [41]. Recently, Bentz and co-workers [42] reported phenotypic switching in *C. auris* when cultured onto CHROMagar *Candida*, on which *C. albicans* and *C. tropicalis* can be relatively reliably identified via a colony color change (*C. albicans*—green, *C. tropicalis*—navy blue). Other clinically relevant *Candida* species, including *C. auris*, will have a pale appearance. Further culturing of *C. auris* on CHROMagar *Candida* led to the description of three predominant colony types: white, pale and sectored (dark purple). No texture changes were observed, as all colonies displayed a smooth and glossy phenotype [42].

The identification of a transition between the three *C. auris* phenotypes shows similarities to the white-opaque transition in *C. albicans*, which was proposed to be tri-stable, due to the presence of an intermediate gray phenotype [36]. In addition, phenotypic switching in *C. albicans* is regulated by the master regulator *Wor1* [41]. *C. auris* possesses three potential genes homologous with *WOR1* that could control phenotypic switching in *C. auris*, similar to that of *C. albicans* [42]. The more closely related species *C. lusitanae* also seems to possess a tri-stable switching system, which is highly associated with antifungal resistance

and filamentation [43, 44], indicating that phenotypic switching could contribute to antifungal resistance in *C. auris* as well. Taken together, phenotypic switching in *C. auris* could have important implications for its pathogenicity, virulence, sexual reproduction and ability to colonize distinct host niches, which warrants further investigation.

#### Attacking the Host: Lytic Enzyme Production and Secretion

The production of extracellular hydrolytic enzymes has been recognized as an important virulence trait contributing to the pathogenicity of *Candida* species. Proteinases are by far the most commonly virulence-associated enzymes. In addition, also hemolysins, lipases and phospholipases seem to play a crucial role.

Secreted aspartyl proteinases (SAPs) are one of the most significant extracellular enzymes produced in *C. albicans*. Classically, these enzymes were considered to play a role in the degradation of host tissue to provide nutrients for pathogen propagation. However, in recent decades, proteinases have also been associated with cell wall maintenance, the formation of polymicrobial biofilms, adhesion to external protective barriers of the host, deregulation of the complement system, inactivation of host antimicrobial peptides, evasion of the immune responses and the induction of inflammatory mediator release from host cells (as reviewed by others [45, 46]). In *C. albicans*, SAPs are encoded by a family of ten genes. Especially *SAP4*, *SAP5* and *SAP6* seem to play an important role in virulence, since inhibition of the production of these proteins greatly attenuates *C. albicans* pathogenicity [47].

Many other pathogenic *Candida* species possess SAP genes, including *C. dubliniensis*, *C. parapsilosis* and *C. tropicalis* [45, 46]. Genomic analysis showed *C. tropicalis* to have at least four SAP genes. *C. parapsilosis* possesses as many as 14 potential SAP genes, and the genome of *C. dubliniensis* encodes eight members of the aspartyl proteinase family. In the *C. auris* genome (strain Ci 6684), hydrolases are the largest group of enzymes (42%), in which four orthologues of SAPs have been found [21]. In vitro studies confirmed that *C. auris* is able to secrete proteinases, although in a strain-dependent manner [31, 48, 49]. Comparing SAP activity of *C. auris* and *C. albicans* demonstrated high activities at 25 °C,

37 °C and 40 °C. Interestingly, *C. auris* SAP activity at 42 °C was much higher than that of *C. albicans*, indicating *C. auris* may be able to maintain its pathogenicity at higher temperatures [31].

Other molecules such as the pore-forming toxins hemolysins are needed to achieve host colonization. Secretion of hemolysins is considered to promote survival within the mammalian host by allowing assimilating iron from the hemoglobin-heme group [50]. Many common pathogenic *Candida* species display hemolysin activity, including *C. albicans*, *C. dubliniensis*, *C. glabrata* and *C. tropicalis* [50–53]. Hemolysin production seems to be higher in strains isolated from hospital infections compared to those from environmental sources, indicating this trait to be an important virulence factor [5]. In *C. auris*, hemolysin production was also observed [49]. Nonetheless, this was a single isolate only and variation in hemolysin activity between the different *C. auris* clades still needs to be evaluated.

Another important group of lytic enzymes, the lipases and phospholipases, have also been shown to be produced by *C. auris* [48, 49]. Lipases play an important role in biofilm formation, host cell damage and immune evasion [54, 55]. For example null mutants of *C. parapsilosis* unable to secrete lipases were more efficiently ingested and killed by macrophage-like cells [54]. Moreover, lipase disruptant mutants of *C. albicans* and *C. parapsilosis* were shown to be significantly less virulent in a rat model of neonatal candidiasis [56]. A comparative analysis of multiple pathogenic *Candida* species demonstrated that *C. albicans* has the highest phospholipase activity [57]. Although *C. auris* has a similar amount of lipase encoding genes in its genome as *C. albicans* [24], the ability to produce phospholipases seems to be reduced and strain-dependent [48]. Production and secretion of a wide variety of enzymes is likely to contribute to rapid spread of *C. auris*. However, further investigation is warranted to reveal to which extent these enzymes are involved in *C. auris* virulence and pathogenicity.

#### Defense Against Antifungals: Intrinsic Resistance of Biofilms

Many microbes are found in biofilm ecosystems. The biofilm forms a structured microbial community encased in a matrix of exopolymeric material. Biofilm



formation by *Candida* species is of particular interest, since its association with increased antifungal resistance and protecting cells within the biofilm from the host immune system [58]. This makes biofilm formation an important virulence trait for *Candida* species linked to excess morbidity and mortality [59, 60].

Multiple studies showed that *C. albicans* has the highest biofilm forming ability compared to other pathogenic *Candida* species [32, 57, 61]. Also *C. auris* strains form significantly reduced biofilms compared to *C. albicans* [32, 48]. On the other hand, biofilm formation by *C. auris* is significantly higher than that by *C. glabrata* [32]. Phenotypical observation also demonstrated *C. auris* biofilms being intermediate to that of *C. albicans* and *C. glabrata*. The *C. auris* biofilms mostly consists of budding yeasts and occasionally pseudohyphae embedded in a limited amount of extracellular matrix. *C. albicans* biofilms are formed by densely packed hyphae and yeast cells embedded in extracellular matrix, whereas *C. glabrata* forms a thin biofilm with yeast cells only, lacking extracellular matrix [32].

Transcriptomic analysis of temporally developing *C. auris* biofilms demonstrated adhesin-related glycosylphosphatidylinositol (GPI)-anchored cell wall genes (*CSA1*, *IFF4*, *PGA26* and *PGA52*) are upregulated during every stage of biofilm production. Moreover, when the biofilm develops into more intermediate and mature stages the expression of genes encoding efflux pumps of the ABC transporter (*CDR1*, *SNQ2* and *YHD3*) and major facilitator superfamily (*MDR1* and *RDC3*) is increased [62].

Adherence is essential for biofilm formation. Agglutinin-like sequence (ALS) proteins, especially Als3, play a key role in *C. albicans* adherence [63, 64]. Interestingly, only two orthologues of members of the ALS proteins (*Als1* and *Als5*) were found in the transcriptome of temporally developing *C. auris* biofilms [62]. Moreover, comparative genomic studies also revealed a highly reduced number of ALS and other proteins belonging to the adhesin and integrin gene families in *C. auris* compared to *C. albicans* [21, 24]. These findings seem to be in contrast with a recent study conducted by Singh and colleagues (2018) who identified three *C. albicans* Als3 protein homologs in *C. auris* through bioinformatic and structural homology modeling [65]. Even more interesting, antibodies targeting *C. albicans* Als3 protein were also able to bind in vitro to five *C. auris* strains,

obtained from different clades. This showed the universal presence of Als3 homologs on the cell surface of *C. auris*. In addition, sera containing anti-Als3 antibodies significantly inhibit biofilm formation of *C. auris*, indicating an essential role of Als3 in biofilm formation similar to *C. albicans* [65]. However, the underrepresentation of the ALS protein family in *C. auris* implies that *C. auris* rely on additional adherence mechanisms. Likely, the GPI-anchored cell wall proteins play a role in *C. auris* adherence. For example, *Csa1* and *Iff4* have been associated with adherence in *C. albicans* [62, 66]. Despite *Iff4* being associated with adherence to silicone catheter [66], the lack of ALS genes seems to impair adherence of *C. auris*, since *C. albicans* exhibits a significantly higher ability to adhere to silicone elastomer of catheters [48].

*Candida* biofilms show intrinsic resistance against antifungals. Several mechanisms were proposed to contribute to this resistance: (1) the high cell density within the biofilm; (2) decreased growth rate and nutrient limitation; (3) sequestration of drugs by the extracellular matrix (ECM); (4) the high expression of resistance genes, especially those encoding efflux pumps; and (5) the presence of ‘persister’ cells [58]. Also *C. auris* biofilms display lower susceptibility against antifungals, including caspofungin, micafungin and amphotericin B [32, 62]. The limited amount of ECM and reduced biomass of *C. auris* biofilms, compared to *C. albicans*, suggests other mechanisms to be more important for this reduced susceptibility [32, 48]. Nonetheless, sequestration of fluconazole by the ECM has recently been observed in *C. auris* biofilms [67].

High expression of efflux pumps also seems to play an important role, as a number of genes encoding these pumps are significantly upregulated in *C. auris* biofilms [62]. Moreover, inhibition of these pumps by an efflux pump inhibitor increases susceptibility of *C. auris* biofilms to fluconazole 2 to 8 times [62]. In addition, the ABC transporter *Cdr1* was shown to be more highly expressed among azole-resistant isolates of *C. auris* [34, 68]. Deletion of this transporter dramatically decreased the minimum inhibitory concentration for all clinically available triazoles as much as 128-fold [68].

## *Candida auris* Stress Tolerance and Persistence as a Nosocomial Pathogen

*Candida auris* is reported to be thermotolerant, being able to grow at temperatures up to 42 °C [16, 69, 70]. The ability of *C. auris* isolates to grow at elevated temperatures appears to be similar to *C. albicans*, but the closer related *C. haemulonii* seems to lose viability at temperatures above 37 °C [70]. In addition, *C. auris* shows excellent salt tolerance compared to other *Candida* species (e.g., *C. albicans*, *C. duobushaemulonii*, *C. haemulonii*, *C. parapsilosis* and *C. tropicalis*). When grown in saline Sabouraud broth (10% wt/vol NaCl), *C. glabrata* was the only species other than *C. auris* to have observable growth [71]. Improved tolerance to thermic and osmotic stresses compared to other *Candida* species is likely to contribute to the pathogenicity of *C. auris*. However, the molecular mechanism behind this increased tolerance remains elusive.

*Candida auris* not only displays excellent stress tolerance, it also has the alarming ability to persistently colonize the human host and the hospital environment. Nosocomial *C. auris* outbreaks have been reported in hospitals all around the world, some of them persisting up to 16 months [12, 72, 73]. Moreover, *C. auris* supposedly has the ability to cause low-grade disease years after colonization. Heath et al. [69] described a case of *C. auris* sternal osteomyelitis in a patient who was colonized by *C. auris* 3 years prior clinical disease manifestation. In addition, a case report from Belgium reported about a patient who was persistently colonized by echinocandin-resistant *C. auris* up to 18 months after its first detection [74]. This demonstrates that *C. auris* infection can progress slowly for > 12 months.

Although the ecological niches of *C. auris* remain unidentified, environmental sampling within the hospital environment has demonstrated *C. auris* to colonize and persist on abiotic surfaces such as bedding material, floors, sinks, as well as human skin, ears and nasal cavities [12, 71, 73, 75]. After a period of 7 days, *C. auris* recovery from dry or moist surfaces was shown to be similar to that of other clinically relevant *Candida* species, including *C. albicans*, *C. glabrata* and *C. parapsilosis* [75]. Additionally, it was shown that *C. auris* is able to remain viable for at least 14 days on a plastic health care surface, as measured by colony forming units (CFU) [71]. However,

compared to *C. parapsilosis*, a species known to colonize plastic and skin, this was strongly reduced, since *C. parapsilosis* remains viable for at least 28 days on this kind of surfaces. On the other hand, using an esterase activity assay to measure individual cells for viability demonstrated that *C. auris* cells were viable 2 weeks longer than initially measured by CFU. This suggests that *C. auris* cells enter a viable but non-culturable state after 14 days [71]. Even though *C. auris* seems to be able to colonize plastic health care surfaces, this fungus shows a weak adherence ability to catheter surfaces made of silicone elastomer, compared to *C. albicans* [48]. Implying a reduced number of catheter-associated candidiasis caused by *C. auris* relative to *C. albicans*.

Several (review) articles report suboptimal efficacy of commonly used hospital environment disinfectants against *C. auris* as one of the factors contributing to its persistence within the hospital environment. However, multiple original studies show high efficacy of a plethora of commercially available disinfectants against *C. auris* compared to *C. albicans* [76–78]. Only quaternary ammonium-based disinfectants seem to be significantly less effective against *C. auris*, but also against *C. albicans* and *C. glabrata* [78]. However, most studies did not assess the efficacy of disinfectants on hospital surfaces and other materials commonly found in hospital settings, such as fabrics and polymer. To establish effective infection prevention protocols preventing the transmission of *C. auris* via contaminated surfaces, examination of *C. auris* disinfection on this kind of surfaces is needed. To this end, several qPCR assays that have recently been developed for the direct detection of *C. auris* can be applied, either by using DNA extracted directly from environmental swabs, overnight enrichment broth cultures or colonies [71, 79–83].

As mentioned before, the integrin and adhesin gene families seem to be underrepresented in the *C. auris* genome and transcriptome. Therefore, *C. auris* likely employs different strategies to adhere and persist on abiotic surfaces. The aggregation of cells into large and difficult to disperse clusters may be one of these strategies that promote persistence in the hospital environment. Aggregation seems to be strain-dependent and caused by the failure to release daughter cells after budding. This results in large aggregates that cannot be physically disrupted [84]. Although aggregating strains display significantly less virulence

compared to non-aggregating strains, it might be that this morphology could have a role in protecting *C. auris* from detergents used to clean hospital environments. Further studies are required to unravel the molecular mechanism of persistence and adhesion in *C. auris*.

### *Candida auris* Virulence In Vivo

Various experimental in vivo models have been developed to test virulence exhibited by *Candida* species [85]. The mouse model is widely used as a representative of the mammalian model. Despite mice being an excellent model to study pathogenicity and virulence of fungi, use of this model is facing ethical conflict and economic issues [86]. Since the innate immune system is evolutionary conserved in insects, invertebrate organisms such as *Drosophila melanogaster* [85], *Caenorhabditis elegans* [87, 88] *Tenebrio molitor* [89] and *Galleria mellonella* [86] are gaining interest as models for studying virulence traits of *Candida* species and host response against *Candida* infections.

Comparative analysis of virulence exhibited by *C. auris* strains and most other common pathogenic *Candida* species in the *G. mellonella* model demonstrated *C. auris* to be significantly more virulent than most of the tested species, with non-aggregative strains showing even similar pathogenicity as *C. albicans* (in terms of kinetics of larval death and number of larvae killed) [30]. This is remarkable, since pathogenicity of *Candida* species in *G. mellonella* was previously reported to be directly related to the development of hyphal filaments or pseudohyphae [84, 90]. Dissection of larvae infected with *C. auris* showed no hyphal or pseudohyphal formation by *C. auris*, indicating a different mechanism of pathogenicity in *G. mellonella* [30]. Sherry et al. [32] also confirmed non-aggregative *C. auris* strains to exhibit similar or even higher virulence than *C. albicans* in the *G. mellonella* model. Since biofilm formation is a key driver of *C. albicans* pathogenicity [59] and non-aggregative *C. auris* strains were shown to have better biofilm forming capacity compared to aggregative strains, this could be one of the factors explaining the difference in virulence between these strain types of *C. auris* [32].

Invertebrate models lack an adaptive immune system and are not suitable for organ colonization assessments. Moreover, some models (e.g., *C. elegans*

and *D. melanogaster*) are not functional at the mammalian body temperature of 37 °C. Therefore, the validity of these models in reference to the human host is negotiable. To this end, a mouse model is likely to more closely represent the human situation.

A comparative study of *C. auris* virulence and its sibling *C. haemulonii* was performed in an immunosuppressed mouse model [70]. Mice infected with *C. auris* rapidly died with only 20% surviving after 5 days. In contrast, *C. haemulonii* isolates showed no virulence at all, with 100% of the mice surviving 12 days post-inoculation. Despite similar virulence observed in the *G. mellonella* model, death of mice infected with *C. albicans* was significantly faster than that of mice infected with *C. auris* [70]. Another comparative study of *C. auris* virulence, using an immunocompetent mouse model, showed similar results, with high virulence of *C. auris*, *C. albicans* and *C. glabrata* isolates. Although *C. haemulonii* did exhibit virulence, this was still significantly lower compared to all other *Candida* species tested. On the other hand, no significant differences in mice survival were found between *C. auris* with *C. albicans* and *C. glabrata* [91]. Nonetheless, a single *C. auris* isolate, obtained from a Chinese fungemic patient, was shown to have significant reduced virulence compared to *C. albicans* in both a mouse model and in *G. mellonella*. In the mouse model, all mice survived 14 days after infection with this *C. auris* isolate, where mice infected with *C. albicans* died by the 6th day post-infection [31]. This indicates the outcome of in vivo virulence assays with *C. auris* is highly strain specific. Fungal burden assays showed highest fungal load of *C. auris* and *C. albicans* to be detected in kidneys, spleen, liver and lungs, respectively [70, 91]. Interestingly, histopathological analysis showed yeast cell aggregates in kidneys of *C. auris*-infected mice, distinct from tissue invasive hyphae observed in kidneys of *C. albicans*-infected mice [70]. Altogether, compared to *C. albicans*, *C. auris* seem to be less virulent in the mouse model. This could be caused by the inability of *C. auris* to produce invasive hyphal filaments.

The immune response to *C. auris* was investigated using a zebrafish model of invasive candidiasis [92]. First in vitro experiments, using human neutrophils showed co-culturing of *C. auris* with human neutrophils had no effect on the initial fungal burden and *C. auris* even replicated beyond the initial inoculum. In contrast, neutrophils inhibited *C. albicans* growth with



75%. Moreover, in mixed cultures, neutrophils preferentially engulfed and killed *C. albicans* over *C. auris*. The strong antifungal response to *C. albicans* results in only 5% survival, while *C. auris* was strikingly resistant to neutrophil killing. The zebrafish model revealed that approximately 50% less neutrophils were recruited in response to *C. auris* infection when compared to *C. albicans*. Fluorescence microscopy revealed neutrophils failed to form neutrophil extracellular traps (NETs) in *C. auris*-infected zebrafish. In contrast, these antimicrobial structures were readily formed when zebrafish were challenged with *C. albicans* [92].

Taken together, the data provided by both mammalian and invertebrate in vivo models suggest *C. auris* is significantly more virulent than most other non-*albicans* *Candida* species. Even compared to *C. albicans*, the virulence of *C. auris* was not always significantly lower [91] and in the *G. mellonella* model non-aggregating strains were even shown to have higher virulence [30, 32]. The higher virulence of *C. auris* in invertebrate models could be explained by its ability to evade neutrophil attack [92]. Invertebrates only have an innate immune system and thus are relying mainly on granulocytes (neutrophil-like cells) as their defense against fungal infection [89], suggesting this system fails to kill *C. auris* effectively.

## Conclusions and Research Outlooks

*C. auris* recently emerged as a global nosocomial pathogen associated with multidrug resistance and high mortality rates. However, the origin of this unprecedented emergence remains unclear. Genomic analyses revealed *C. auris* possesses many genes associated with virulence and reduced antifungal susceptibility, including genes encoding secreted aspartyl proteases, lipases, phospholipases, hemolysins and drug efflux pumps [7, 21, 24]. Nonetheless, many genes are still uncharacterized and further investigation is required to understand the molecular mechanism responsible for the high pathogenicity and antifungal resistance of this pathogen.

Although the molecular mechanisms remain mostly unknown, this review shows that *C. auris* expresses many important virulence traits, such as biofilm formation, phenotypic switching, secretion of lytic enzymes and high stress tolerance, that are likely to

have contributed to its emergence as a nosocomial pathogen. Moreover, *C. auris* seems to have the alarming ability to persistently colonize health care environments and human host, despite the reduced amount of adhesins in its genome.

An important limitation of virulence analysis in *C. auris* seems to be the high variability between strains, especially between strains of different clades [7]. For example, secretion of lytic enzymes was demonstrated to be strain specific [48]. More interestingly, antifungal susceptibility also seems to be highly heterogeneous, since multidrug-resistant strains [7] as well as totally susceptible strains have been isolated [31]. This shows that future studies should be careful when extrapolating findings to all isolates of the species and preferably incorporate multiple strains of different clades in their experimental setup.

Animal studies demonstrated *C. auris* is highly virulent and capable of inducing systemic infection and mortality to a much higher extent than other non-*albicans* *Candida* species, such as the closely related *C. haemulonii* or other potential multidrug-resistant yeast *C. glabrata*. Although strain specific, *C. auris* approaches the pathogenicity of *C. albicans*, which could be partially explained by the observation made that *C. auris* is able to evade the innate immune response and production of NETs by human neutrophils. In contrast, *C. albicans* is highly susceptible to killing by the innate immune system [30, 92].

In conclusion, *C. auris* expresses many important virulence traits, including traits that are well characterized in other *Candida* species, and seemingly unique traits, such as the ability to evade the innate immune system and persistently colonize the skin of human host. This together with the high propensity to develop resistance to multiple antifungals likely contributed to its emergence as a nosocomial pathogen.

## Compliance with Ethical Standards

**Conflict of interest** The authors declare that they have no conflict of interest.

**Research Involving Human Participants and/or Animals** This article does not contain any studies with human participants or animals performed by any of the authors.

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